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DIVISION OF CHEMISTRY

Composition and Digestibility of the
Chloroform Extract of Hays
and Fodders



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COMPOSITION AND DIGESTIBILITY OF THE CHLOROFORM EXTRACT OF HAYS AND FODDERS

BY

G. S. FRAPS, PH. D., Chemist.

J. B. RATHER, M. S., Assistant Chemist.

It was proposed to follow the examination of the ether extract of hays and foddors (published as Bulletin No. 150), with a study of the alcoholic extract. Preliminary studies showed, however, that on dilution of the alcoholic extract with water, and extraction of it with ether, appreciable percentages of material were secured, which partly consisted of unsaponifiable and saponifiable matter, together with chlorophyll. As previous studies (Bulletin 150) had shown that the feeding stuffs contain substances difficultly soluble in cold ether, but easily soluble in chloroform, some preliminary tests were made by extracting the material (previously extracted with ether) with chloroform. As these tests showed appreciable quantities of chloroform-soluble substances, the investigation here reported was undertaken.

QUANTITY OF THE CHLOROFORM EXTRACT.

The quantity of the chloroform extract is given in Table 1, for comparison with the percentages of ether extract, secured by the extraction with ether which precedes the chloroform extraction. The chloroform extract is, on an average, 0.80 per cent compared with 1.85 per cent for the ether extract, or 43 per cent. The comparative amounts are close with some of the hays, such as alfalfa, para grass and Bermuda hay.

CONSTITUENTS OF THE CHLOROFORM EXTRACT.

The chloroform extract from the green hays and foddors was highly colored, and clearly contained chlorophyll. The residue left after saponification and extraction of the unsaponified material, was likewise highly colored. No attempt was made in the investigation of the ether extract to separate the coloring matter from the fatty acids.

In the work on the chloroform extract it was found that if the alkali solution remaining after extraction of the unsaponified were made acid with acetic acid and extracted with petroleum ether, the coloring matter remained in the aqueous solution, and the fatty acids secured were nearly colorless. On acidification of the aqueous solution with hydrochloric acid and extraction with ethyl ether, a third product was secured, which was highly colored when the source of the chloroform extract was green hays or foddors. This method is based upon statements in Beilstein III, page 657, according to which, alkachlorophyll may be purified by extracting the aqueous solution of the sodium salt, dissolved in water and acidified with acetic acid, with ether, and on

TABLE 1.—Comparative Percentages of Chloroform Extract and of Ether Extract.

Period.	Description.	Total Chloroform Extract.	Total Ether Extract.
3	Alfalfa hay	0.98	1.28
12	Bermuda hay	0.84	1.52
9	Buffalo grass hay	0.70	1.25
6	Burr clover	1.58	1.98
17	Corn shucks	0.31	0.59
16	Guam grass	0.84	1.78
4	Johnson grass	0.85	1.29
10	Johnson grass hay	0.91	1.38
15	Kaffir fodder	1.04	1.99
11	Millet	0.55	1.53
5	Oat hay	0.53	2.12
14	Para grass hay	0.58	0.86
13	Peanut hay	0.71	8.17
7	Rice straw (Japan)	0.57	1.47
18	Rice straw (Honduras)	0.82	1.24
2	Sorghum hay	0.78	1.47
8	Vetch hay	1.05	1.59
	Average	0.80	1.85

the further statement that alkachlorophyll is insoluble in petroleum ether.

By means of this procedure, which is described in detail below, the chloroform extract was separated into three constituents:

- A. Unsaponifiable material.
- B. Colorless saponified material.
- C. Colored saponified material.

Quantities of five hundred grams each of the hays, fodders or excrements were separated into these three constituents, and they were then subjected to further study.

Unsaponified Material.—The acetyl compounds of the unsaponified material from three products were prepared and recrystallized twice from alcohol. The mother liquors were combined and evaporated. The acetyl numbers of the products are as follows:

PROPERTIES OF CRYSTALLIZED ACETYL PRODUCTS.

	Saponification Number.	Melting Point.
4240 Excrement, Johnson grass hay.....	112.0	69-70°
4261 Excrement, peanut hay	124.0	71-73°
4665 Excrement, rice straw.....	115.0	72-73°

The acetyl numbers of the crystals are near to that of myricyl alcohol, which is 116.4. This corresponds to the crystals separated from the ether extract of burr clover.

The saponification numbers of the acetyl compounds in the mother liquors also correspond to some extent with the saponification numbers of the mother liquors from the acetyl compounds from the ether extract (Bulletin No. 150). The former were extracted from the excrements, the latter from the feeds.

SAPONIFICATION NUMBER OF ACETYL COMPOUNDS OF MOTHER LIQUORS.

	Chloroform extract.	Ether extract.
4240 Johnson grass hay.....	176.9	163.1
4261 Peanut hay	196.7	168.7
4665 Rice straw	203.8	190.0

It appears probable that the unsaponified material of the chloroform extract consists of wax alcohols or is derived from waxes, which were not completely extracted during the previous extraction with ether.

Fatty Acids.—The fatty acids, which were nearly colorless, had the following neutralization numbers. The corresponding values for the ether extract are also given.

	Chloroform extract.	Ether extract.
3279 Excrement, alfalfa	87.1	165
4240 Excrement, Johnson grass.....	127.8	140
4261 Excrement, peanut hay	171.2	205
4665 Excrement, rice straw.....	160.5	169

The error may be several units, on account of the small quantity of

material used. The saponified material from the ether extract contained chlorophyll, and was green in color, which interfered with the titration.

There are considerable differences between the fatty acids of the ether extract and of the chloroform extract; yet we must allow for the presence of the chlorophyll in the ether extract and for the fact that the fats and oils are easily soluble in ether, which would account for a part of the differences. It is quite probable that the fatty acids are in the form of waxes not easily soluble in ether; it is also possible that a portion comes from the decomposition of other substances soluble in chloroform.

Colored Saponified Extract.—The colored saponified extract was prepared by a method which could separate chlorophyll from the fatty acids. From rice straw, it was yellow in color. The following are some estimations of nitrogen in these products; 0.7 gm. substance was used:

3279Per cent nitrogen	1.42
3700Per cent nitrogen	0.72
3877Per cent nitrogen	0.26
3223Per cent nitrogen	0.58
4240Per cent nitrogen	0.56
4261Per cent nitrogen	0.64
4665Per cent nitrogen	0.38
Average		0.65

The chlorophyll present in this saponified extract would probably be present as phylloatain, containing 12.0 per cent nitrogen. If we assume that all the nitrogen is present in this compound, the colored saponified extract would contain, on an average, 5.41 per cent; the maximum amount present would be 11.53 per cent. The bulk of the product must, therefore, consist of other substances than chlorophyll. This conclusion is also in accord with a number of other determinations of nitrogen made directly on the total chloroform extract.

QUANTITATIVE SEPARATION.

The method for the quantitative separation of the constituents of the chlorophyll extract is described in detail below. The results of the separation of the constituents of the hays and fodders are given in Table 2. Table 3 shows the percentage composition of the extracts, based upon the total chloroform extract as 100.

A comparison of the percentage composition of the ether extract and of the chloroform extract, from the hays and fodders, shows great differences.

	Unsaponifiable.	Total saponifiable.	Loss.
Ether extract	58	36	6
Chloroform extract.....	25	55	20

The chloroform extract contains much less unsaponifiable matter and, when both colorless and colored saponified are taken together to be the

TABLE 2.—Composition of Chloroform Extract in Percentage of Hays and Fodders.

Period.	Description.	Total Chloroform Extract.	Unaponifiable.	Colorless saponifiable.	Colored saponifiable.	Loss.
3	Alfalfa hay.....	0.98	0.18	0.16	0.42	0.22
12	Bermuda hay.....	0.84	0.29	0.19	0.20	0.16
9	Buffalo grass hay.....	0.70	0.16	0.10	0.28	0.16
6	Burr clover.....	1.58	0.35	0.36	0.49	0.38
17	Corn shucks.....	0.31	0.11	0.08	0.15	*0.03
16	Guam grass.....	0.84	0.09	0.13	0.35	*0.27
4	Johnson grass.....	0.85	0.39	0.26	0.22	0.02
10	Johnson grass hay.....	0.91	0.23	0.11	0.41	0.16
15	Kaffir fodder.....	1.04	0.21	0.11	0.43	0.29
11	Millet.....	0.55	0.20	0.14	0.10	0.11
5	Oat hay.....	0.53	0.13	0.07	0.20	0.13
14	Para grass hay.....	0.58	0.07	0.11	0.21	0.19
13	Peanut hay.....	0.71	0.15	0.20	0.22	0.14
7	Rice straw (Japan).....	0.57	0.18	0.11	0.18	0.10
18	Rice straw (Honduras).....	0.82	0.16	0.11	0.30	0.25
2	Sorghum hay.....	0.78	0.16	0.10	0.25	0.27
8	Vetch hay.....	1.05	0.29	0.38	0.37	0.01
	Average.....	0.80	0.20	0.16	0.28	0.17

*Gain.

TABLE 3.—Percentage Composition of Chloroform Extract of Hays and Fodders.

Period.	Description.	Total chloroform extract.	Unaponifiable.	Colorless saponifiable.	Colored saponifiable.	Loss.
3	Alfalfa hay.....	0.98	18.	16.	43.	22.
12	Bermuda hay.....	0.84	35.	23.	24.	19.
9	Buffalo grass hay.....	0.70	23.	14.	40.	23.
6	Burr clover.....	1.58	22.	23.	31.	24.
17	Corn shucks.....	0.31	35.	26.	48.	*10.
16	Guam grass.....	0.84	11.	15.	42.	32.
4	Johnson grass.....	0.85	46.	31.	26.	* 2.
10	Johnson grass hay.....	0.91	25.	12.	45.	18.
15	Kaffir fodder.....	1.00	20.	11.	41.	28.
11	Millet.....	0.55	36.	25.	18.	20.
5	Oat hay.....	0.53	25.	13.	38.	25.
14	Para grass hay.....	0.58	12.	19.	36.	33.
13	Peanut hay.....	0.71	21.	28.	31.	20.
7	Rice straw (Japan).....	0.57	32.	19.	32.	18.
18	Rice straw (Honduras).....	0.82	20.	13.	37.	30.
2	Sorghum hay.....	0.78	21.	13.	32.	35.
8	Vetch hay.....	1.05	28.	36.	35.	1.
	Average.....	0.80	25.↓	20.↓	35.	20.

*Gain.

total saponifiable, it contains much more saponifiable. If, however, we consider that the colorless saponifiable matter of the chloroform extract corresponds to the saponifiable matter of the ether extract, we have 36 per cent of the ether extract to compare with 20 per cent in the chloroform extract. This assumption is justified, to a certain extent, for we know that the ether extract contains fats and oils (if present) and that the chloroform extract will contain non-fats. The chief differences between the ether extract and the chloroform extract would then consist in much larger quantities of colored saponified matter (not liberated by acetic acid and dissolved by petroleum ether) and a much higher percentage of loss. The higher percentage of loss may be in part due to the loss inherent in working on such small quantities; it may also be due to the solution of substances in water, not subsequently extracted by the ether.

The conclusion is, that while chloroform dissolves some waxes or wax alcohols not dissolved by the previous extraction with ether, it also dissolves materials not soluble easily in ether; which are neither waxes, wax alcohols nor fatty acids, and which contain only a comparatively small percentage of chlorophyll, but are somewhat related to chlorophyll in their behavior towards acetic acid and solvents.

DIGESTIBILITY OF THE PRODUCTS.

The digestibility of the chloroform extract and its products is worked out in the tables given at the end of this Bulletin. Table 4 shows the average digestibility of the chloroform extract of the various hays and fodders. A comparison of the average percentage of digestibility of the ether extract and the chloroform extract is made below:

COEFFICIENTS OF DIGESTIBILITY OF CONSTITUENTS OF EXTRACTS.

	Chloroform extract.	Ether extract.
Total	45.7	41.9
Unsaponifiable	21.4	29.1
Saponifiable (total).....	66.4
Saponifiable (colorless).....	45.4
Saponifiable (colored).....	39.5
Loss (water soluble).....	75.7

The average digestibility of the total chloroform extract and of the total ether extract agree closely. There is also some agreement between the individual hays and fodders, though not always close. The greatest difference is peanut hay, of which the ether extract is much more readily digested, but this contains more fats and oils than ordinary fodders, on account of the presence of the peanuts.

The average digestibility of the unsaponifiable matter are also not so very far apart, though the unsaponifiable in the chloroform extract is less easily digested. The saponifiable matter is much less easily digested from the chloroform extract than from the ether extract. It appears probable that the fatty acids extracted by the chloroform are present in the feed in a different form of combination from those in the ether extract,

TABLE 4.—Average Digestibility of Constituents of Chloroform Extract of Hays and Fodders.

Period Number.	Description.	Total chloroform extract.	Unsaponifiable.	Colorless saponifiable.	Colorless saponifiable.	Loss.
3	Alfalfa hay.....	52.9	13.5	55.3	48.9	91.0
12	Bermuda hay.....	50.9	33.2	70.8	20.1	93.5
9	Buffalo grass hay.....	61.4	31.3	57.5	37.5	68.8
17	Corn shucks.....	36.0	18.6	44.9	44.1	0.0
16	Guam grass.....	37.2	0.0	26.9	42.9	56.5
4	Johnson grass.....	40.7	36.2	48.1	52.9	0.0
10	Johnson grass hay.....	47.9	0.0	10.3	68.9	93.8
15	Kaffir fodder.....	59.4	15.5	19.3	64.6	100.0
11	Millet.....	63.0	28.8	64.3	0.0	61.4
5	Oat hay.....	28.6	20.0	18.5	23.1	94.0
14	Para grass hay.....	45.2	0.0	31.8	34.9	100.0
13	Peanut hay.....	22.9	0.0	38.8	9.1	87.5
7	Rice straw (Japan).....	16.7	20.9	54.8	0.0	44.3
18	Rice straw (Honduras).....	43.9	41.2	45.7	18.8	62.5
2	Sorghum hay.....	41.1	0.0	0.0	53.3	85.4
8	Vetch hay.....	67.6	68.1	68.4	72.3	0.0
	Average.....	45.7	21.4	45.4	39.5	75.7

which compounds are much less easily digested. The loss, probably consisting of material soluble in water, is much more easily digested than other constituents of the feed.

METHOD FOR DETERMINATION OF CONSTITUENTS OF CHLOROFORM EXTRACT OF HAYS AND FODDERS.

The following method was used in this work:

Purification of Alcohol.—Dissolve 1.5 grams silver nitrate in 3 c.c. water, add to 1000 c.c. alcohol and shake. Dissolve 3 grams caustic potash in about 25 c.c. warm alcohol and add to the alcohol. Shake, allow the precipitate to settle, pour off the alcohol and distill. Do not let stand longer than three days before pouring off.

Caustic Soda.—Dissolve 40 grams caustic soda, pure by alcohol, in 500 c.c. purified alcohol, filter and dilute to 1000 c.c. Titrate 10 c.c. with N/5 hydrochloric acid. Ten c.c. should require 25 c.c. or more of the acid.

Extraction.—Use 10 grams of the substance which has previously been extracted for sixteen hours with ether. Extract for sixteen hours with redistilled chloroform, transfer to a tared flask, evaporate, dry, and weigh.

Saponification.—Transfer to 500 c.c. Jena erlenmeyer with hot chloroform and evaporate off the solvent on water bath. Use 5 c.c. of the alcoholic soda for each $\frac{1}{2}$ gram of the chloroform extract, and 20 c.c. alcohol. Saponify by boiling with a reflux condenser for five hours, shaking gently from time to time, and using great care that the caustic comes in contact with all the fat which may be present in the flask. Add 0.25 grams sodium bicarbonate for every 5 c.c. of alcoholic soda used, and stir well. Evaporate off all alcohol in steam bath. Dry thirty minutes in a water oven.

Extraction of Unsaponified.—Heat the soap prepared above with 50 c.c. petroleum ether, which distills below 80° C., for twenty minutes on water bath under reflux condenser with shaking. Add 25 c.c. water and heat for thirty minutes longer. Draw off the clear petroleum ether and filter through a filter paper previously extracted with ether, if it contains any suspended matter. If the solution is not clear, wash with 25 c.c. of 1 to 1 alcohol. By the use of a pear shaped separatory funnel and allowing the extract to stand, the insoluble matter will usually separate and fall to the bottom of the funnel, where it can be separated and returned to the original mixture. In case it is necessary to filter and use the alcohol wash, evaporate the alcohol washings in a porcelain dish after adding the products of extraction of the filter with alcohol and ether, and return to the soap solution by means of hot water and a steel spatula, before extracting the soap solution with ether. Extract twice with 50 c.c. petroleum ether by heating thirty minutes as above, wash with water three times to remove traces of soap and inorganic substances, returning the wash water to the flask containing the soap. Heat the extracted soap with 50 c.c. ethyl ether as before, separate the clear ether, and wash three times with 15 c.c. water. Allow any emulsion to go back into the flask with the soap. Return the washing to the flask also. Extract with ether in this way

four times. If any emulsion is then present, heat till the ether is all gone. Make a fifth extraction and evaporate separately as a test of completeness of the extraction. If this extraction weighs more than 1 per cent of the original chloroform extract, continue the extraction with ether until it is less than that amount. Put the ethyl ether extracts in the flask with the petroleum ether extracts and evaporate during the progress of the extraction. After completion of the ether extraction, evaporate off the rest of the petroleum ether and ethyl ether, adding alcohol to aid in the removal of the petroleum ether if necessary. Transfer to a tared 100 c.c. erlenmeyer by means of hot chloroform. Evaporate and dry to constant weight.

Correction for Fatty Acids in Unsaponifiable.—Heat unsaponifiable for 15 minutes with 20 c.c. of N/5 hydrochloric acid and 100 c.c. water. Let cool and pour off the water, through a filter if necessary. Heat to boiling with 50 c.c. water, let cool and pour off again. The substance usually sticks to the flask, and the use of a filter is not necessary. Wash four times with water, dissolve in alcohol, and titrate with N/5 caustic soda and phenolphthalein, making a blank test on the alcohol. Calculate to palmitic acid.

Extraction of Fatty Acids.—Heat the soap solution, after the removal of the unsaponified, on a steam bath to remove ether in solution. Heat until the ether is all gone. Cool and acidify with acetic acid, testing with litmus paper. Extract with 50 c.c. petroleum ether three times by shaking the slightly warmed mixture in a separatory funnel and combine the extracts in a 500 c.c. erlenmeyer flask. Extract a fourth time as described in the removal of the unsaponified, wash with 2x15 c.c. water, dry and weigh separately. This extraction should weigh less than 1 per cent of the original chloroform extract. Wash the combined extracts with 2x50 c.c. of water to remove inorganic salts. Return to the erlenmeyer and evaporate to dryness, transfer to a tared 100 c.c. erlenmeyer with hot chloroform, evaporate and dry to constant weight.

Extraction of the Chlorophyll, etc.—Acidify the residue from the above extraction further with hydrochloric acid, and add a few cubic centimeters of alcohol. Extract with ethyl ether just as described in the extraction of the fatty acids. Wash with water and complete as described in the extraction of the fatty acids.

References.—Acetic acid acid method for chlorophyll, Beilstein III, 657, "Alkachlorophyll $C_{52}H_{57}N_7O_7$. . . (is) difficultly soluble in absolute ether, easily in alcohol, insoluble in water . . . and ligroin."

Chloroform Extract of Feeds and Excrements.

Number.	Description.	Total.	Unsaponified.	Colorless saponified.	Colored saponified.	Loss.
3224	Sorghum hay.....	0.78	0.16	0.10	0.25	0.27
3258	Excrement, sheep 1.....	1.41	0.59	0.34	0.36	0.12
3277	Alfalfa hay, sheep 1.....	0.98	0.18	0.16	0.42	0.22
3279	Excrement, sheep 2.....	1.30	0.44	0.22	0.61	0.03
3280	Excrement, sheep 3.....	1.19	0.36	0.17	0.58	0.08
3281	Excrement, sheep 4.....	1.13	0.38	0.14	0.52	0.09
3587	Johnson grass hay.....	0.85	0.39	0.26	0.22	*0.02
3589	Excrement, sheep 1.....	1.09	0.54	0.31	0.21	0.03
3590	Excrement, sheep 3.....	1.05	0.60	0.28	0.25	*0.08
3591	Excrement, sheep 4.....	1.01	0.41	0.25	0.19	0.16
3595	Oat hay.....	0.53	0.13	0.07	0.20	0.13
3597	Excrement, sheep 1.....	1.13	0.47	0.17	0.47	0.02
3609	Burr clover.....	1.58	0.35	0.36	0.49	0.38
3623	Excrement, sheep 2.....	2.26	1.09	0.37	0.76	0.04
3649	Vetch hay.....	1.05	0.29	0.38	0.37	0.01
3700	Excrement, sheep 1.....	0.91	0.25	0.32	0.28	0.06
3625	Rice straw.....	0.57	0.18	0.11	0.18	0.10
3877	Excrement, sheep 2.....	0.72	0.23	0.06	0.41	0.02
3878	Excrement, sheep 3.....	1.10	0.27	0.10	0.49	0.24
3879	Excrement, sheep 4.....	0.84	0.25	0.11	†
3883	Buffalo grass hay.....	0.70	0.16	0.10	0.28	0.16
3885	Excrement, sheep 2.....	0.87	0.26	0.10	0.41	0.10
4238	Johnson grass hay.....	0.91	0.23	0.11	0.41	0.16
4240	Excrement, sheep 1.....	1.26	0.63	0.23	0.35	0.05
4241	Excrement, sheep 2.....	1.19	0.63	0.29	0.31	*0.04
4247	Millet.....	0.55	0.20	0.14	0.10	0.11
4249	Excrement, sheep 1.....	0.80	0.34	0.12	0.24	0.10
4252	Bermuda hay.....	0.84	0.29	0.19	0.20	0.16
4254	Excrement, sheep 1.....	0.78	0.30	0.13	0.35	0.0
4255	Excrement, sheep 3.....	0.85	0.46	0.09	0.29	0.01
4259	Peanut hay.....	0.71	0.15	0.20	0.22	0.14
4261	Excrement, sheep 1.....	1.64	0.62	0.37	0.60	0.05
4277	Para grass.....	0.58	0.07	0.11	0.21	0.19
4279	Excrement, sheep 1.....	0.58	0.23	0.14	0.25	*0.04
4546	Kaffir fodder.....	1.04	0.21	0.11	0.43	0.29
4548	Excrement, sheep 4.....	1.29	0.49	0.19	0.61	0.0
4549	Excrement, sheep 5.....	1.09	0.51	0.33	0.25	0.0
4552	Guam grass.....	0.84	0.09	0.13	0.35	0.27
4554	Excrement, sheep 1.....	1.11	0.24	0.20	0.42	0.25
4557	Corn shucks.....	0.31	0.11	0.08	0.15	*0.03
4559	Excrement, sheep 1.....	0.52	0.20	0.12	0.23	*0.03
4560	Excrement, sheep 4.....	0.44	0.24	0.10	0.17	*0.07
4663	Rice straw.....	0.82	0.16	0.11	0.30	0.25
4665	Excrement, sheep 1.....	0.87	0.18	0.11	0.40	0.18

* Gain. † Lost.

Digestibility of Constituents of Ether Extract of Hays and Fodders.

Grams.	Digestion period.	Laboratory No.	Description.	Total extract.	Unsaponifiable.	Colorless saponifiable.	Colored saponifiable.	Loss.
4800 1560	2	3224 3258	Sorghum hay eaten.....	37.5	7.7	4.8	12.0	12.0
			Sheep No. 1 excreted.....	22.0	9.2	5.3	5.6	1.9
				15.5	0.0	0.0	6.4	11.1
				41.1	0.0	0.0	53.3	85.4
4400 1511	3	3277 3280	Alfalfa hay eaten.....	43.1	7.9	7.0	18.5	9.7
			Sheep No. 3 excreted.....	19.6	6.6	3.3	9.2	0.5
				23.5	1.3	3.7	9.3	9.2
				54.5	16.5	52.9	50.3	94.8
4400 1667	3	3277 3279	Alfalfa hay eaten.....	43.1	7.9	7.0	18.5	9.7
			Sheep No. 2 excreted.....	21.7	7.3	3.7	10.2	0.5
				21.4	0.6	3.3	8.3	9.2
				49.6	7.6	47.2	44.9	94.8
4400 1732	3	3277 3281	Alfalfa hay eaten.....	43.1	7.9	7.0	18.5	9.7
			Sheep No. 4 excreted.....	19.6	6.6	2.4	9.0	1.6
				23.5	1.3	4.6	9.5	8.1
				54.5	16.5	56.7	51.4	83.5
3490 1720	4	3587 3589	Johnson grass hay eaten.....	29.6	13.6	9.0	7.7	0.0
			Sheep No. 1 excreted.....	18.7	9.3	5.3	3.6	0.5
				10.9	4.3	3.7	4.1	0.0
				36.8	31.6	41.1	53.2	0.0
3129 1476	4	3587 3590	Johnson grass eaten.....	26.6	12.2	8.1	6.9	0.0
			Sheep No. 3 excreted.....	15.5	8.9	4.1	3.7	0.0
				11.1	3.3	4.0	3.2	0.0
				41.7	27.0	49.2	46.4	0.0
3442 1637	4	3587 3591	Johnson grass eaten.....	29.2	13.4	8.9	7.6	0.0
			Sheep No. 4 excreted.....	16.5	6.7	4.1	3.1	2.6
				12.7	6.7	4.8	4.5	-2.6
				43.5	50.0	53.9	59.2	0.0
3827 1281	5	3595 3597	Oat hay eaten.....	20.3	5.0	2.7	7.8	5.0
			Sheep No. 1 excreted.....	14.5	6.0	2.2	6.0	0.3

4000 1096	6	3609 3623	Sheep No. 1 digested	5.8	1.0	0.5	1.8	4.7
			Percentage	28.6	20.0	18.5	23.1	94.0
			Burr clover eaten	63.2	14.0	14.4	19.6	15.2
			Sheep No. 2 excreted	24.7	11.9	4.1	8.3	0.4
3547 1914	7	3625 3877	Sheep No. 2 digested	38.5	2.1	10.3	11.3	14.8
			Percentage	60.9	15.0	71.5	80.3	97.4
			Rice straw eaten	20.2	6.4	3.9	6.4	3.5
			Sheep No. 2 excreted	13.7	4.4	1.1	7.8	0.4
3295 1933	7	3625 3878	Sheep No. 2 digested	6.5	2.0	2.8	0.0	3.1
			Percentage	32.2	31.3	71.8	0.0	88.6
			Rice straw eaten	18.7	5.9	3.6	5.9	3.3
			Sheep No. 3 excreted	21.3	5.2	1.9	9.5	4.6
3430 1912	7	3625 3879	Sheep No. 3 digested	-2.6	0.7	1.7	-3.6	-1.3
			Percentage	0.0	11.9	47.2	0.0	0.0
			Rice straw eaten	19.6	6.2	3.8	6.2	3.4
			Sheep No. 4 excreted	16.1	5.0	2.1	0.0	0.0
4000 1491	8	3649 3700	Sheep No. 4 digested	3.5	1.2	1.7	0.0	0.0
			Percentage	17.9	19.4	44.7	0.0	0.0
			Vetch hay eaten	42.0	11.6	15.2	14.8	0.4
			Sheep No. 1 excreted	13.6	3.7	4.8	4.1	0.9
4000 1701	9	3883 3885	Sheep No. 1 digested	28.4	7.9	10.4	10.7	-0.5
			Percentage	67.6	68.1	68.4	72.3	0.0
			Buffalo grass hay eaten	28.0	6.4	4.0	11.2	6.4
			Sheep No. 2 excreted	14.8	4.4	1.7	7.0	1.7
4000 1512	10	4238 4240	Sheep No. 2 digested	17.2	2.0	2.3	4.2	4.4
			Percentage	61.4	31.3	57.5	37.5	68.8
			Johnson grass hay eaten	36.4	9.2	4.4	16.4	6.4
			Sheep No. 1 excreted	19.1	9.5	3.5	5.3	0.8
4000 1577	10	4238 4241	Sheep No. 1 digested	17.3	0.0	0.9	11.1	5.6
			Percentage	47.5	0.0	20.5	67.7	87.5
			Johnson grass hay eaten	36.4	9.2	4.4	16.4	6.4
			Sheep No. 3 excreted	18.8	9.9	4.6	4.9	0.0
4000 1686	11	4247 4249	Sheep No. 3 digested	17.6	0.0	0.0	11.5	6.4
			Percentage	48.3	0.0	0.0	70.1	100.0
			Millet eaten	22.0	8.0	5.6	4.0	4.4
			Sheep No. 1 excreted	13.5	5.7	2.0	4.0	1.7

Digestibility of Constituents of Ether Extract of Hays and Fodders.—Continued.

Grams.	Digestion period.	Laboratory No.	Description.	Total.	Unsaponifiable.	Colorless saponifiable.	Colored saponifiable.	Loss.
			Sheep No. 1 digested.....	8.5	2.3	3.6	0.0	2.7
			Percentage.....	63.0	28.8	64.3	0.0	61.4
3988 1920	12	4252 4254	Bermuda hay eaten.....	33.5	11.6	7.6	8.0	6.4
			Sheep No. 1 excreted.....	15.0	5.8	2.5	6.7	0.0
			Sheep No. 1 digested.....	18.5	5.8	5.1	1.3	6.4
			Percentage.....	55.2	50.0	67.1	16.3	100.0
3988 2110	12	4252 4255	Bermuda hay eaten.....	33.5	11.6	7.5	8.0	6.4
			Sheep No. 3 excreted.....	17.9	9.7	1.9	6.1	0.2
			Sheep No. 3 digested.....	15.6	1.9	5.6	1.9	6.2
			Percentage.....	46.6	16.4	74.6	23.8	96.9
4000 1335	13	4259 4261	Peanut hay eaten.....	28.4	6.0	8.0	8.8	5.6
			Sheep No. 1 excreted.....	21.9	8.3	4.9	8.0	0.7
			Sheep No. 1 digested.....	6.5	0.0	3.1	0.8	4.9
			Percentage.....	22.9	0.0	38.8	9.1	87.5
3960 2175	14	4277 4279	Para grass eaten.....	23.0	2.8	4.4	8.3	7.5
			Sheep No. 1 excreted.....	12.6	5.0	3.0	5.4	0.0
			Sheep No. 1 digested.....	10.4	0.0	1.4	2.9	7.5
			Percentage.....	45.2	0.0	31.8	34.9	100.0
4000 1409	15	4546 4549	Kaffir fodder eaten.....	41.6	8.4	4.4	17.2	11.6
			Sheep No. 5 excreted.....	15.6	6.9	2.7	8.6	0.0
			Sheep No. 5 digested.....	23.4	1.5	1.7	8.6	11.6
			Percentage.....	56.3	17.9	38.6	50.0	100.0
4000 1433	15	4546 4549	Kaffir fodder eaten.....	41.6	8.4	4.4	17.2	11.6
			Sheep No. 5 excreted.....	15.6	7.3	4.7	3.6	0.0
			Sheep No. 5 digested.....	26.0	1.1	-0.3	13.6	11.6
			Percentage.....	62.5	13.1	0.0	79.1	100.0
4000 1899	16	4552 4554	Guam grass eaten.....	33.6	3.6	5.2	14.0	10.8
			Sheep No. 1 excreted.....	21.1	4.6	3.8	8.0	4.7
			Sheep No. 1 digested.....	12.5	0.0	1.4	6.0	6.1
			Percentage.....	37.2	0.0	26.9	42.9	56.5

3155 1350	17	4557	Corn shucks eaten.....	9.8	3.5	2.5	4.7	0.0	
		4559	Sheep No. 1 excreted.....	7.0	2.7	1.6	3.1	0.0	
				2.8	0.8	0.9	1.6	0.0	
				28.6	22.9	36.0	34.0	0.0	
3200 1691	18	4663	Rice straw eaten.....	26.2	5.1	3.5	9.6	8.0	
		4665	Sheep No. 1 excreted.....	14.7	3.0	1.9	6.8	3.0	
						11.5	2.1	1.6	1.8
						43.9	41.2	45.7	18.8
								5.0	
								62.5	

SUMMARY AND CONCLUSIONS.

(1) Chloroform extracts comparatively large percentages of material from hays and fodders which have previously been extracted with ether.

(2) The chloroform extract contains wax alcohols, fatty acids, chlorophyll and unknown substances.

(3) The chloroform extract is different in quantitative composition from the ether extract, containing less unsaponifiable matter, more saponifiable matter, and with a greater loss. It contains high percentages of substances related in their properties to chlorophyll.

(4) The average digestibility of the total chloroform extract and the total ether extract, are in fairly close agreement.

(5) The unsaponifiable material in the chloroform extract is digested to a less extent than that in the ether extract. The digestibility of both is low.

(6) The saponifiable material of the chloroform extract is digested to a much greater extent than the unsaponifiable material. It is digested to a much less extent than the saponifiable material of the ether extract, which latter contains larger proportions of true fats.

(7) A method is given for separating the chloroform extract into several groups of constituents.